Comparative morphology and ultrastructure of Malpighian tubules of the mute cicada *Karenia caelatata* and the coleopterous spittlebug *Lepyronia coleoptrata* (Hemiptera: Cicadomorpha)

ZHONG Hai-Ying[1,2], ZHANG Ya-Lin[1], WEI Cong[1,*]

(1. Key Laboratory of Plant Protection Resources and Pest Management, Ministry of Education, Entomological Museum, Northwest A&F University, Yangling, Shaanxi 712100, China; 2. State Key Laboratory Breeding Base for Zhejiang Sustainable Pest and Disease Control, Institute of Plant Protection and Microbiology, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China)

Abstract: 【Aim】 In order to better understand the morphology and function of Malpighian tubules of the Cicadomorpha, comparative morphology and ultrastructure of the mute cicada *Karenia caelatata* Distant and the coleopterous spittlebug *Lepyronia coleoptrata* (Linnaeus) were investigated. 【Methods】 The Malpighian tubules of adult *K. caelatata* and *L. coleoptrata* were observed using both light and transmission electron microscopies. 【Results】 The results show that except the anterior-most segment that takes part in the formation of the filter chamber, each tubule outside the filter chamber of both *K. caelatata* and *L. coleoptrata* is morphologically differentiated into four regions, *i. e.*, intermediate duct, proximal segment, distal segment, and terminal segment. Apical parts of the terminal segments in *K. caelatata* are attached to the ileum, with the posterior-most ends united in pairs and enveloped by a connetcive tissue shortly before arriving at the rectum, whereas the corresponding parts in *L. coleoptrata* terminate as separated rods and closely attached to the rectum. Ultrastructurally, cells of the tubules in both species possess basal infoldings and apical microvilli. Cells of the proximal segment in *K. caelatata* possess secretory vesicles, secretory granules, rough endoplasmic reticulum and urospherites. In *L. coleoptrata*, cells of the proximal segment possess abundant secretory vesicles, and cells of the distal segment contain oval vesicles which appear to fuse with the basal plasma membrane. The microorganisms observed in the proximal and distal segments of *K. caelatata* are possibly symbiotic bacteria, while those resided in the intermediate duct of *L. coleoptrata* are likely to be pathogenic. 【Conclusion】 The similarities and differences in morphological characteristics and ultrastructure of Malpighian tubules in the two Cicadomorpha representatives, *K. caelatata* and *L. coleoptrata*, not only support the sister relationship of Cicadoidea and Cercopoidea, but also improve our understanding of the functional differentiation of Malpighian tubules in the Cicadomorpha. The discovery of microorganisms in the Malpighian tubules of the two species is informative to the future study of coevolution between the Cicadomorpha and related bacteria and to the biological control of related pests.

Key words: Auchenorrhyncha; *Karenia caelatata*; *Lepyronia coleoptrata*; excretory system; symbiotic bacteria; transmission electron microscopy; light microscopy

1 INTRODUCTION

The Malpighian tubules and the rectum form the major excretory system of insects. The blindly ending tubules emerge from the junction of the midgut and hindgut and usually lie freely in the hemocoel of most insects (Gillott, 2005). They only exist in the form of papillae in Diplura, Strepsiptera and Protura, but are devoid in Collembola and aphids (Hemiptera, Sternorrhyncha) (Chapman, 1998). In many coleopterans, some larval hymenopterans and neuropterans and nearly all larval lepidopterans, the distal ends of the Malpighian tubules are apposed closely to the rectum surface and enclosed by a peripnephric membrane, thus form a modified arrangement, *viz.*, the cryptonephric complex (Gillott, 2005). The Malpighian tubules of thrips *Aeolothrips intermedius* and *Frankliniella occidentalis*

基金项目：国家自然科学基金项目(31572302)

作者简介：钟海英，女，1984 年生，青海西宁人，博士，助理研究员，研究方向为昆虫系统性与生物多样性，E-mail：zhy8085@nwsuaf.edu.cn

[*] 通讯作者 Corresponding author, E-mail：congwei@nwsuaf.edu.cn

收稿日期 Received：2017-02-14；接受日期 Accepted：2017-06-12

(Thysanoptera) are adhered to the hindgut, indicating a probable cryptonephridial role (Dallai *et al.*, 1991; Conti *et al.*, 2010). However, in the Cicadomorpha of Hemiptera, the distal portions of the Malpighian tubules are attached to the rectum, but do not form the so-called 'cryptonephric complex': the anterior-most part is taking part in the formation of a water shunting complex structure, known as "filter chamber" in the Auchenorrhyncha (Cheung and Marshall, 1973). The Malpighian tubules are one-cell thick and comprise a single layer of epithelial cells surrounding the lumen (Chapman, 1998). Ultrastructurally, the apical border of the tubule cells bears well-developed microvilli, thus forms a brush border; the basal plasma membrane is highly invaginated (Gillott, 2005). The cell structure of Malpighian tubules is closely related to transport of substances, *e. g.*, isosmotic filtrate (primary urine) is produced in the Malpighian tubules (Gullan and Cranston, 2005).

The infraorder Cicadomorpha is a dominant lineage of Auchenorrhyncha (Hemiptera), with approximately 35 000 described species worldwide, classified into three superfamilies: Cicadoidea (cicadas), Membracoidea (leafhoppers, sharpshooters and treehoppers), and Cercopoidea (spittlebugs or froghoppers) (Dietrich, 2005). The major relationships within this infraorder are supported as "Membracoidea + (Cicadoidea + Cercopoidea)" (Rakitov, 2002; Li *et al.*, 2015). Insects of the Cicadomorpha use their sucking mouthparts to feed solely on plant phloem or xylem sap (Moran *et al.*, 2005). Plant sap contains some levels of carbohydrates, mainly in the form of sucrose, but poor in nitrogenous compounds such as amino acids, vitamins, or cofactors (Redak *et al.*, 2004; Noda *et al.*, 2012; Ishii *et al.*, 2013). Their nutrition supply is related to the widespread mutualistic symbiotic bacteria in their alimentary canals and the associated Malpighian tubules (Douglas, 2006).

To date, there are limited documents on the Malpighian tubules in cicadas and spittlebugs. Licent (1912) reported that the proximal portion of the Malpighian tubules in nymphal cicadas possesses a short inflated region. Marshall (1964a, 1964b, 1965) investigated the nymphal Malpighian tubules of several tube-building spittlebugs at the cytological and functional levels. Later, Marshall (1966) performed a histochemical study on the Malpighian tubules of three nymphal spittlebugs (*Aeneolamia varia saccharina*, *Philaenus leucophthalmus spumarius* and *Clovia lineatocollis*), and indicated that large amounts of acid mucopolysaccharide are contained in the proximal segments of the tubules. Marshall and Cheung (1974) conducted ultrastructural and cytochemical investigations on the Malpighian tubules of two adult cicadas (*Gaeana maculata* and *Cryptotympana mimica*) and four nymphal spittlebugs (*Cosmoscarta abdominalis*, *Philaenus spumarius*, *Philagra parva* and *Machaerota coronata*), focusing on the transportation of water and ion. Recently, Rakitov (2002) studied the proximal and distal segments of Malpighian tubules in Cicadoidea and Cercopoidea, and supposed that the peculiar adaptations of nymphal cicadas and spittlebugs possess a common origin. More recently, the distal segments of Malpighian tubules of both adults and nymphs in Cicadoidea and Cercopoidea were particularly investigated by Li *et al.* (2015).

Such morphological and/or ultrastructure studies of one or two regions of the Malpighian tubules provided valuable information for understanding the functions and evolution of the Cicadomorpha. However, a more detailed illustration of the Malpighian tubules is desirable. Herein, we investigated the gross morphology and ultrastructure of the Malpighian tubules of the mute cicada *Karenia caelatata* and the coleopterous spittlebug *Lepyronia coleoptrata*, with special reference to the distribution of organelles and microorganisms hosted there.

2 MATERIALS AND METHODS

2.1 Species collection

Adults of *K. caelatata* (Cicadoidea) and *L. coleoptrata* (Cercopoidea) were collected in July 2014 from Yangling, Shaanxi Province, China.

2.2 Observation of the general structure of Malpighian tubules under light microscopy

Living insects (at least ten individuals for each species) were anesthetized and dissected in phosphate buffered saline (PBS, 0.2 mol/L, pH 7.2). The general structure of the Malpighian tubules of all the dissected insects was observed using a stereoscopic zoom microscope (Motic SMZ168). Photographs were taken using a scientific digital micrography system equipped with an auto-montage imaging system and a Qimaging Retiga 2000R digital camera (CCD).

2.3 Ultrastructure observation of Malpighian tubules under transmission electron microscopy (TEM)

The Malpighian tubules were fixed in 2.5% glutaraldehyde (phosphate buffered saline, 0.1 mol/L, pH 7.2) for 24 h at 4℃, rinsed in phosphate buffered saline (PBS, 0.1 mol/L, pH 7.2) and postfixed in 1% osmium tetroxide for 2 h. After the dehydration in a graded series of ethanol (30%, 50%, 70%, 80%, 90% and 100%) and 100% acetone, the materials were infiltrated in a mixture of Epon 812 and acetone, and were embedded in Epon 812. Ultra-thin sections were stained with uranyl acetate and lead citrate, and observed under a JEM-1230 TEM at 80 kV.

The terminology of the Malpighian tubule structure follows that of Rakitov (2002).

3 RESULTS

3.1 General structure of Malpighian tubules in *K. caelatata* and *L. coleoptrata*

Four Malpighian tubules arise from the junction of the midgut and hindgut in both *K. caelatata* and *L. coleoptrata*. Each tubule is morphologically differentiated into five distinct segments. The anterior-most segment takes part in the formation of the filter chamber and is packaged in the filter chamber, which is also referred to as the filter chamber segment. The tubule outside the filter chamber is morphologically differentiated into four regions: 1) the smooth intermediate duct (id) or intermediate segment, connecting anteriorly with the filter chamber segment and posteriorly with the proximal segment; 2) the proximal segment (ps), which is longer than the intermediate duct; 3) the distal segment (ds), which is extremely long and thick; and 4) the undulated terminal segment (ts), which is thinner than the distal segment (Fig. 1: A, B).

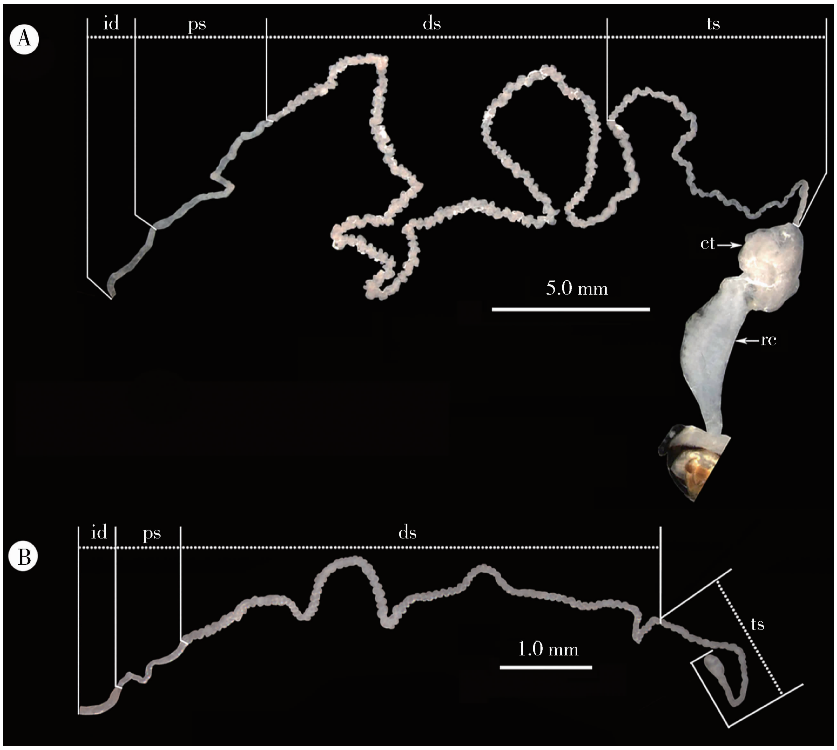

Fig. 1 Partition of an individual Malpighian tubule of *Karenia caelatata* (A) and *Lepyronia coleoptrata* (B) under light microscopy
Arrowhead indicates part of the filter chamber. id: Intermediate duct; ps: Proximal segment; ds: Distal segment; ts: Terminal segment; ct: Connective tissue encompassing part of the terminal segment; rc: Rectum.

In *K. caelatata*, the apical parts of the terminal segments are attached to the ileum, with the posterior-most ends united in pairs and enveloped by a connective tissue shortly before reaching to the rectum (Fig. 1: A). However, in *L. coleoptrata*, the corresponding parts terminate as separate rods and closely attached to the rectum (Fig. 1: B). Ultrastructurally, cells of the four tubule regions outside the filter chamber of both species rest on a basal lamina, but exhibit great differences.

3.2 Ultrastructure of Malpighian tubules in *K. caelatata*

The thin connective tissue attached on the outer surface of the intermediate duct is penetrated by tracheoles (Fig. 2: A). Longitudinal muscles externally enveloping intermediate duct cells are visible. Two types of cells standing on a thick basal lamina (approximately 0.8 μm thick) are recognized, both possessing well-developed apical microvilli and infoldings of basal plasma membrane.

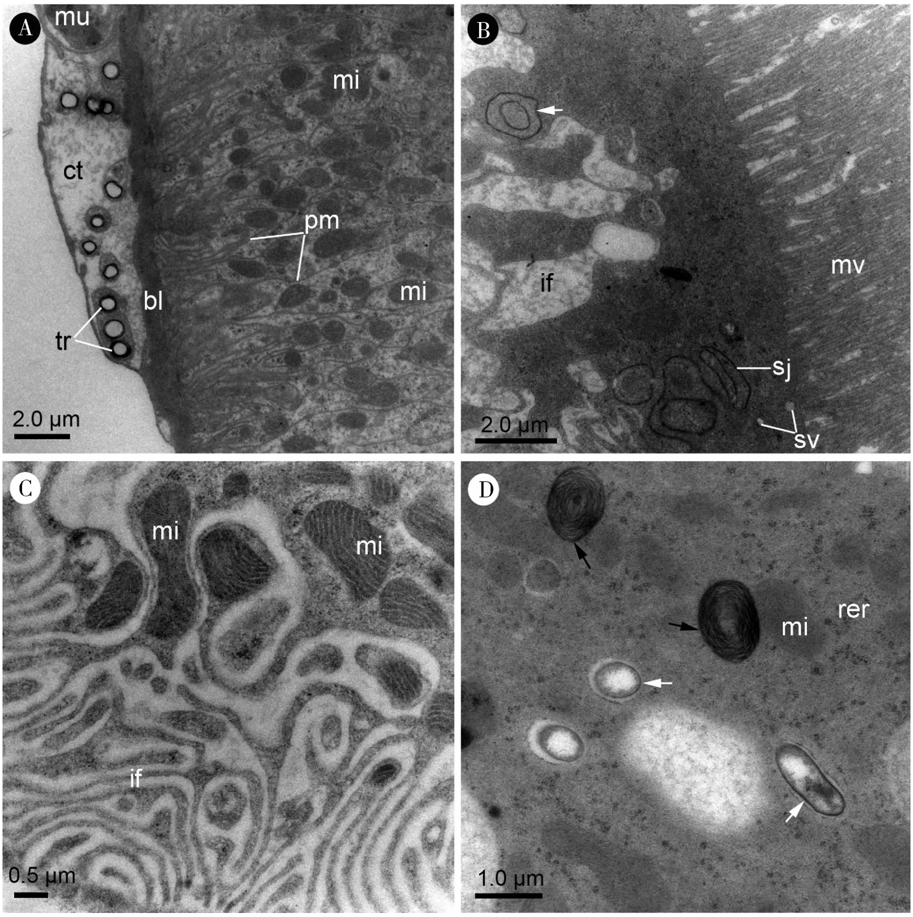

Fig. 2 TEM micrographs of the Malpighian tubules of *Karenia caelatata*

A: Peripheral portion of the first cellular type of the intermediate duct, showing well-developed infoldings of the basal plasma membrane (pm) associated with mitochondria (mi). Tracheoles (tr) penetrated to the connective tissue (ct). B: Basal infoldings (if) of the second cellular type of the intermediate duct are very wide. The apical microvilli (mv) are long and densely-packed. The cytoplasm contains secretory vesicles (sv). Septate junctions (sj) join the cells. Arrow indicates a portion of junctional cell contact. C: Basal part of the cell of the proximal segment, showing shallow basal infoldings (if) associated with mitochondria (mi). The mitochondria have evident cristae. D: Cytoplasm of the proximal segment, showing mitochondria (mi), scattered elements of rough endoplasmic reticulum (rer) and urospherites (as indicated by black arrows). White arrows indicate microorganisms scattered in the cytoplasm. bl: Basal lamina; mu: Longitudinal muscles.

The basal infoldings of the first type of cells are extremely narrow and associated with mitochondria (Fig. 2: A); while those of the second type of cells are very wide and devoid of mitochondria (Fig. 2: B). Near the base of the microvilli, scattered secretory vesicles and septate junctions are observed (Fig. 2: B).

Cells of the proximal segment are characterized by shallow basal infoldings associated with mitochondria, densely-packed long microvilli, scattered secretory granules, and secretory vesicles filled with granular materials (Figs. 2: C, D; 3: A). The inner membrane of the mitochondrion folds inwards, forming the developed cristae (Figs. 2: C). Scattered elements of rough endoplasmic reticulum are observed throughout the cytoplasm (Figs. 2: D; 3: A). Electron-dense urospherites (about 0.8 μm in diameter) are observed in the cytoplasm (Fig. 2: D). Rod-shaped microorganisms (approximately 0.7 μm in diameter, 1.4 μm in length) with single membrane exist in the cytoplasm (Fig. 2: D).

In the distal segment, cells rest on a thick basal lamina (Fig. 3: B). The basal plasma membrane invaginates into well-developed infoldings associated with mitochondria (Fig. 3: B); the apical border of the cells bears long microvilli (Fig. 3: C). Numerous mitochondria are observed through the cytoplasm (Fig. 3: C). Rod-shaped microorganisms (about 0.5 – 0.9 μm in diameter, 0.8 – 2.2 μm in length) with single-membrane exist throughout the cytoplasm (Figs. 3: C, D; 4: A).

Cells of the terminal segment are similar to those of the distal segment, which are featured by long apical microvilli and well-developed basal infoldings associated with elongated mitochondria. These mitochondria contain numerous cristae and occupy the most area of the cells (Fig. 4: B).

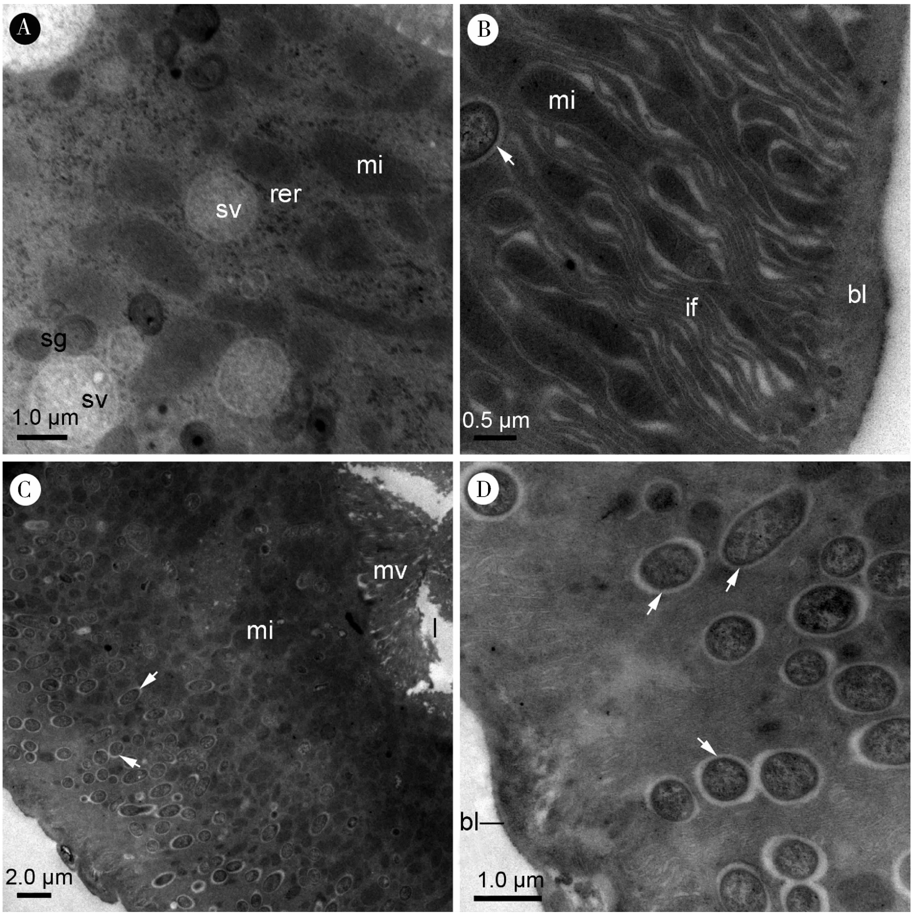

Fig. 3　TEM micrographs of the Malpighian tubules in *Karenia caelatata*

A: Detail of the cytoplasm of the proximal segment, showing mitochondria (mi), secretory granules (sg), secretory vesicles (sv), and scattered elements of rough endoplasmic reticulum (rer). B: Basal infoldings (if) of the distal segment cells are well-developed and associated with mitochondria (mi). Arrow indicates microorganisms. C: Cross-section of the distal segment, showing numerous microorganisms (as arrows indicate) in the cytoplasm. Abundant mitochondria (mi) exist through the cytoplasm. The apical microvilli (mv) are dense and long. D: Detail of a cell of the distal segment. bl: Basal lamina; l: Lumen.

3.3　Ultrastructure of Malpighian tubules in *L. coleoptrata*

Cells of the intermediate duct are featured by developed basal infoldings and densely-packed apical microvilli (Fig. 4: C, D). These infoldings are relatively short, widely and regularly-arranged, in comparison with those in the corresponding region of *K. caelatata* (Fig. 4: C). The microvilli are very long and densely packed (Fig. 4: D). Rod-shaped microorganisms (approximately 0.4 μm in diameter, 1.5 – 2.0 μm in length) with single-membrane occupy the degenerated cytoplasm, leaving very limited space for microorganisms scattered in the intact cytoplasm (Figs. 4: C, D; 5: A). The occurrence of numerous microorganisms seems to result in a degeneration of some cells, which makes it difficult to distinguish other organelles in such cells. Electron-lucent and membrane-bounded vacuoles (about 3.0 μm in diameter), appearing to be formed by fusion of smaller ones, are distributed randomly in the cytoplasm (Fig. 5: B). Profiles of the rough endoplasmic reticulum that exist around the large vacuoles are observed at the apical region of the cells (Figs. 4: D; 5: B).

Cells of the proximal segment are joined by septate junctions. These cells are remarkably distinct from those of other regions examined: they contain abundant secretory vesicles in the cytoplasm, and bear long densely-packed apical microvilli facing the lumen (Fig. 5: C). The secretory vesicles are different in shape and size, and some smaller vesicles appear to fuse to become larger ones (Fig. 5: D). Numerous mitochondria are visible among the secretory vesicles and also in other areas of the cytoplasm (Fig. 5: D). Large oval nucleus with clumps of heterochromatin is also visible in the cytoplasm (Fig. 5: C, D).

Basal plasma membrane of cells of the distal segment forms labyrinths of infoldings associated with mitochondria (Fig. 6: A). Many oval vesicles with

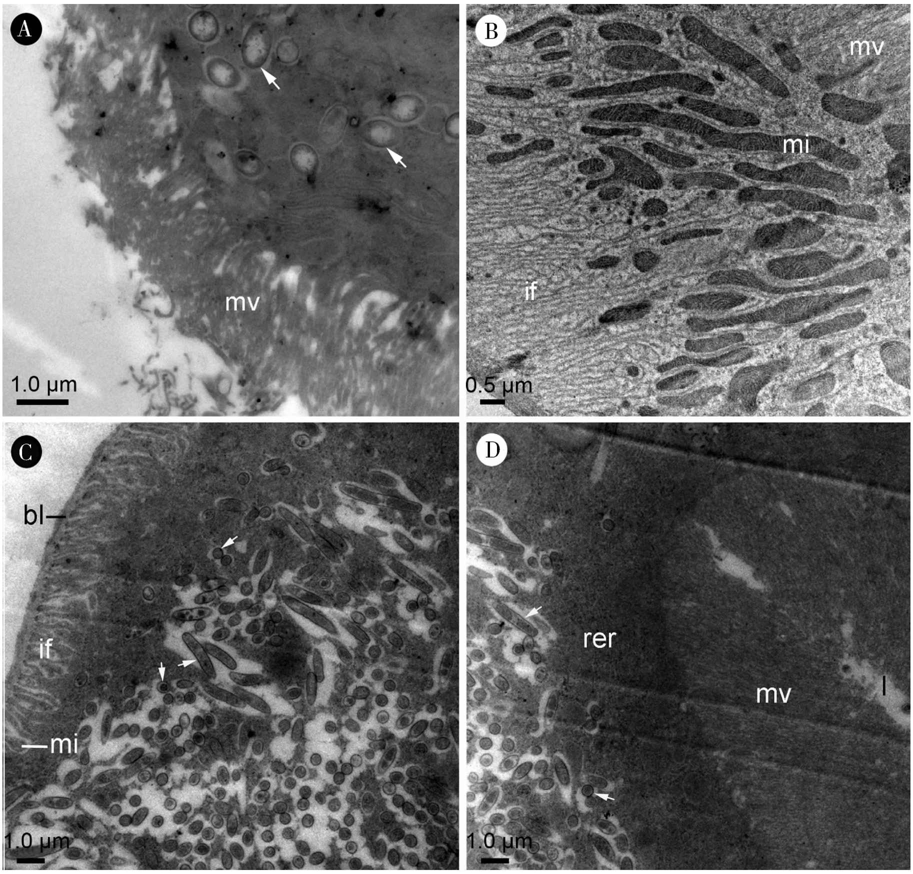

Fig. 4 TEM micrographs of the Malpighian tubules of *Karenia caelatata* and *Lepyronia coleoptrata*

A: Apical part of the cell of the distal segment of *K. caelatata*, showing dense and long microvilli (mv). Microorganisms (as arrows indicate) are observed near the base of the microvilli. B: Basal part of a cell of the terminal segment *K. caelatata*, showing well-developed basal infoldings (if) associated with mitochondria (mi). The mitochondria (mi) with cristae are elongated and different in shape. C: Developed basal infoldings (if) of the intermediate duct cells of *L. coleoptrata* are regularly-arranged. Numerous microorganisms (as arrows indicate) exist in the degenerated cell. D: Transverse section of the intermediate duct of *L. coleoptrata*, showing rough endoplasmic reticulum (rer) exists near the long, dense apical microvilli (mv). Arrows indicate microorganisms. bl: Basement lamina; l: Lumen.

varied size are enclosed by the labyrinth. They appear to be fused with the basal plasma membrane. The cytoplasm is packed with mitochondria, among which the extracellular space associated with dense and long microvilli facing the lumen are observed (Fig. 6: B). Cells of the terminal segment contain lysosomes, numerous mitochondria, and scattered elements of rough endoplasmic reticulum (Fig. 6: D). Cells of this segment are also densely coated with long microvilli at the apical border (Fig. 6: C). The basal infoldings closely associated with mitochondria are relatively sparse (Fig. 6: C).

4 DISCUSSION

Basal infoldings and apical microvilli of the Malpighian tubule cells greatly increase the effective surface area of cells, and efficiently improve the exchange and transport of substances (O′Donnell and Maddrell, 1983). The apical microvilli also play an important part in the maturation of the secretion, including the reabsorption of water, electrolytes and other substances of the original secretion (Nunes and Camargo-Mathias, 2006). Rakitov (2002) supposed that the peculiar adaptations of nymphal cicadas and spittlebugs possess a common origin. In *K. caelatata* and *L. coleoptrata*, the occurrence of well-developed basal infoldings and apical microvilli in cells of all the four regions of the Malpighian tubules outside the filter chamber indicates that effective exchange of ion, transportation of water and modification of secretions occur in all these regions. Abundant mitochondria contained in the infoldings indicate that energy is provided for active transport of certain ions through the tubule wall. The Malpighian tubules of both nymphal cicadas and spittlebugs function to secondary secretion in addition to the excretive function, and become mere excretive organs in adult stage (Li *et al*., 2015). Accordingly, the vesicles found in the proximal segments of *K. caelatata* and *L. coleoptrata* may be the remnants of secretory activity in the larval stage.

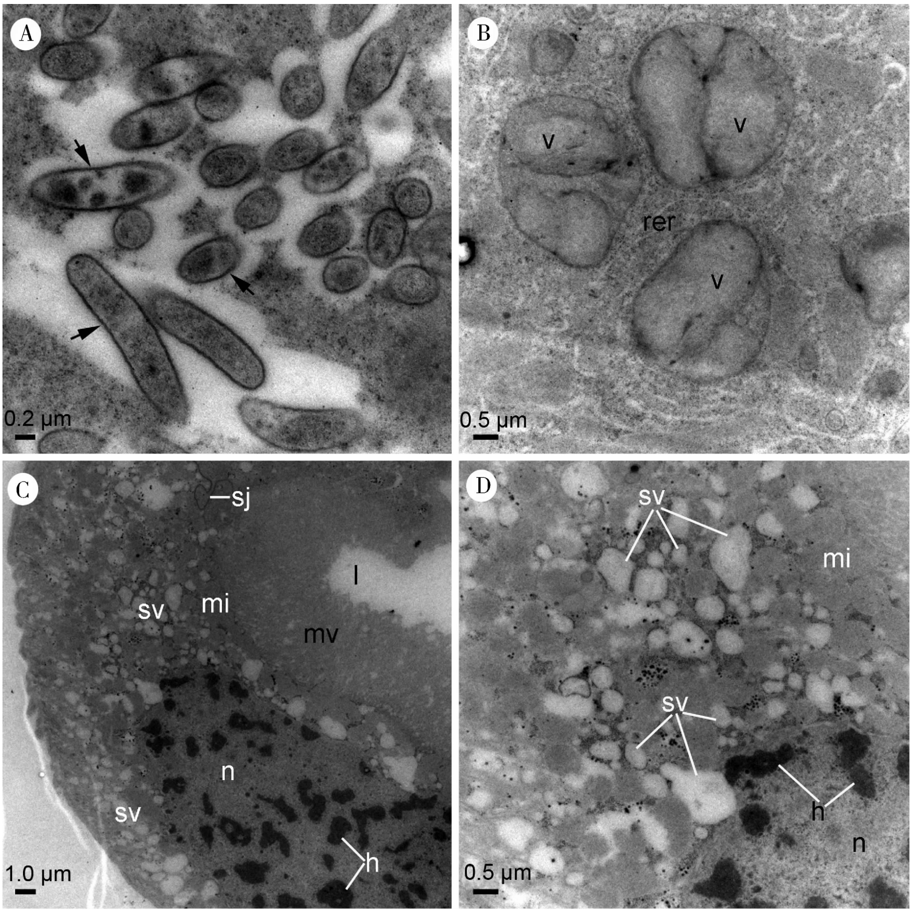

Fig. 5 TEM micrographs of the Malpighian tubules of *Lepyronia coleoptrata*

A: High magnification of microorganisms. Arrows indicate the wall of microorganisms. B: Large vacuoles (v) of the cytoplasm of the intermediate duct appear to be formed by the fusion of smaller ones. Rough endoplasmic reticulum (rer) are visible adjacent to the large vacuoles. C: The cytoplasm of the proximal segment is packed with secretory vesicles (sv) and mitochondria (mi). Microvilli (mv) are long and dense at the apical border of cells. D: Detail of the cytoplasm of the proximal segment, showing secretory vesicles (sv) of varying in size and shape. l: Lumen; h: Heterochromatin; n: Nucleus; sj: Septate junctions.

Urospherites were observed in the proximal segment of Malpighian tubules of *K. caelatata*, which are morphologically similar to those found in the midgut cells of the springtails *Podura aquatica* and *Allacma fusca* (Rost, 2006; Rost-Roszkowska *et al.*, 2007), the flesh-fly *Sarcophaga bullata* (Radford and Misch, 1971), and the mite *Anystis* sp. (Wright and Newell, 1964). These urospherites play an important role in the maintenance of ionic balance and in excretion (Humbert 1978, 1979). Furthermore, they are involved in the resistance of insects to chemical pollution by fixing toxic elements such as heavy metals (Humbert, 1978). Whether the urospherites observed in the proximal segment of Malpighian tubules of *K. caelatata* are also present in the alimentary canal remains unknown, and their function await further investigation.

Microorganisms are widespread in sap-feeding insects, especially in the suborder Auchenorrhyncha of Hemiptera, which was colorfully described as a "fairyland of symbioses" (Baumann *et al.*, 2013). They colonize in some tissues and organs such as the salivary glands (Zhong *et al.*, 2013a), reproductive systems and fat body (Sacchi *et al.*, 2008), alimentary canal (Chen *et al.*, 2006; Zhong *et al.*, 2013b), Malpighian tubules (Crotti *et al.*, 2010; Zhong *et al.*, 2013b; Li *et al.*, 2015), and particularly, the bacteriomes (McCutcheon *et al.*, 2009a, 2009b). Microorganisms play an important role in nutritional supplements (Moran, 2007; Gündüz and Douglas, 2009), manipulation of host's reproduction (Min and Benzer, 1997; Dobson *et al.*, 1999, and prevention from parasitism and pathogens (Moran *et al.*, 2008). The existence of microorganisms in cells of the Malpighian tubules of insects is probably due to the following factors: a suitable environment to inhabit, such as the presence of acidic pH, the availability of partly digested food coming from midgut, and the acquisition of excretory products from Malpighian tubules (Crotti *et al.*, 2010). In our study, many rod-shaped and single-membraned microorganisms were observed in the

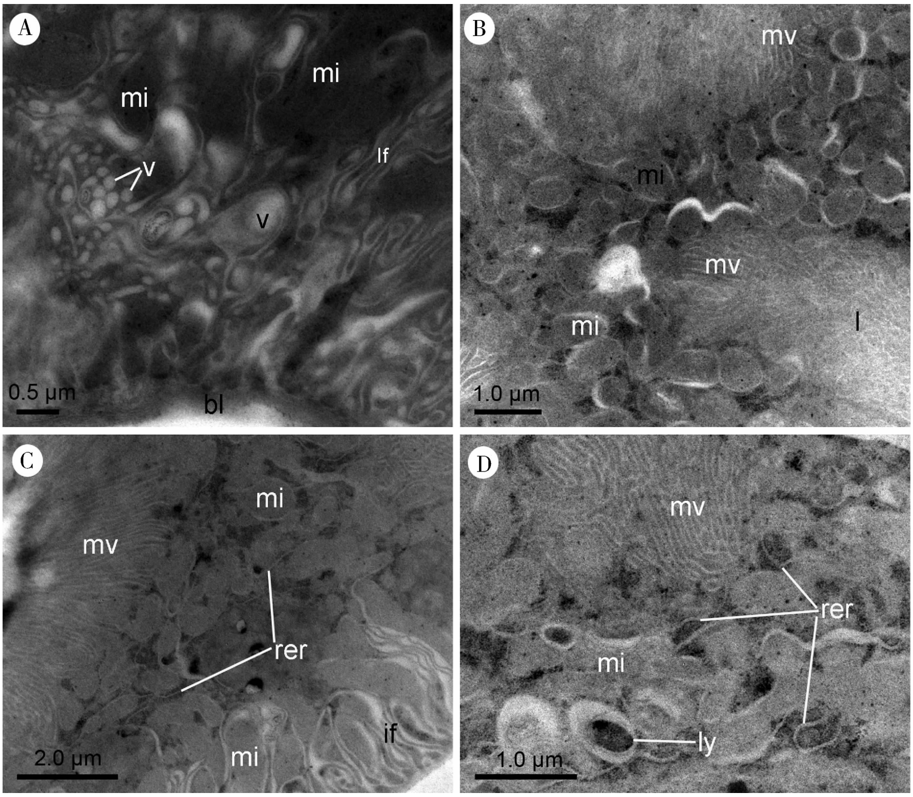

Fig. 6 TEM micrographs of the Malpighian tubules of *Lepyronia coleoptrata*

A: Basal labyrinths of infoldings (if) of the distal segment are associated with mitochondria (mi). Oval vesicles (v) of varying size are surrounded by the labyrinths. B: Numerous mitochondria (mi) are contained in the cytoplasm of the distal segment. Extracellular space (l) associated with long dense microvilli (mv). C: Transverse section of the terminal segment, showing sparse basal infoldings (if) associated with mitochondria (mi). Apical microvilli (mv) are long and densely packed. The cytoplasm contains many mitochondria (mi) and scattered elements of rough endoplasmic reticulum (rer). D: The cytoplasm of the terminal segment contains lysosomes (ly) that scatter among the mitochondria (mi) and sparse rough endoplasmic reticulum (rer). Extracellular space (l) associated with long microvilli (mv). bl: Basement lamina; l: Lumen.

Malpighian tubules of *L. coleoptrata* and *K. caelatata*, respectively. These microorganisms appear to be of different types with respect to diameter, length, endoplasm concentration and residing location. Numerous microorganisms observed in the unbroken cells of the Malpighian tubules in *K. caelatata* are morphologically similar to those presented in the salivary glands of *K. caelatata* (Zhong *et al.*, 2013a), indicating that they are probably symbiotic bacteria. Abundant microorganisms observed in the degenerated cells of the intermediate duct of *L. coleoptrata* are possibly pathogenic. The identity and functions of these microorganisms are worthy of investigation in the future.

In summary, the morphological characteristics of Malpighian tubules in the two Cicadomorpha representatives supply useful information to support the phylogenic relationship that the Cicadoidea is homologous with Cercopoidea. Additionally, they are informative for improving our understanding of the functional differentiation of Malpighian tubules in this infraorder. The discovery of microorganisms in the Malpighian tubules of the two species, *K. caelatata* and *L. coleoptrata*, provides useful information for the further investigation of coevolution between the Cicadomorpha and related bacteria and to the biological control of related pests.

References

Baumann P, Moran NA, Baumann LC, 2013. Bacteriocyte-associated endosymbionts of insects. In: Rosenberg E, DeLong EF, Lory S, Stackebrandte E, Thompson F eds. The Prokaryotes. Prokaryotic Biology and Symbiotic Associations. Springer-Verlag, Berlin, Heidelberg. 465-496.

Chapman RF, 1998. The Insects: Structure and Function. 4th ed. Cambridge University Press, Cambridge. 770 pp.

Chen TY, Chu CC, Hu C, Mu JY, Henneberry TJ, 2006. Observations on midgut structure and content of *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Ann. Entomol. Soc. Am.*, 99(5): 917-919.

Cheung WWK, Marshall AT, 1973. Water and ion regulation in cicadas in relation to xylem feeding. *J. Insect Physiol.*, 19(9): 1801-1816.

Conti B, Berti F, Mercati D, Giusti F, Dallai R, 2010. The ultrastructure of Malpighian tubules and the chemical composition of the cocoon of *Aeolothrips intermedius* Bagnall (Thysanoptera). *J. Morphol.*, 271(2): 244-254.

Crotti E, Rizzi A, Chouaia B, Ricci I, Favia G, Alma A, Sacchi L, Bourtzis K, Mandrioli M, Cherif A, Bandi C, Daffonchio D, 2010. Acetic acid bacteria, newly emerging symbionts of insects. *Appl. Environ. Microb.*, 76(21): 6963-6970.

Dallai R, Del Bene G, Marchini D, 1991. The ultrastructure of Malpighian tubules and hindgut of *Frankliniella occidentalis*

(Pergande) (Thysanoptera: Thripidae). *Int. J. Insect Morphol. Embryol.*, 20(4-5): 223-233.

Dietrich CH, 2005. Keys to the families of Cicadomorpha and subfamilies and tribes of Cicadellidae (Hemiptera: Auchenorrhyncha). *Fla. Entomol.*, 88(4): 502-517.

Dobson SL, Bourtzis K, Braig HR, Jones BF, Zhou W, Rousset F, O'Neill SL, 1999. *Wolbachia* infections are distributed throughout insect somatic and germ line tissues. *Insect Biochem. Mol.*, 29(2): 153-160.

Douglas AE, 2006. Phloem-sap feeding by animals: problems and solutions. *J. Exp. Bot.*, 57(4): 747-754.

Gillott C, 2005. Entomology. 3rd ed. Springer, Netherlands. 834 pp.

Gullan PJ, Cranston PS, 2005. The Insects: An Outline of Entomology. Wiley-Blackwell, Boston. 529 pp.

Gündüz EA, Douglas AE, 2009. Symbiotic bacteria enable insect to use a nutritionally inadequate diet. *Proc. R. Soc. B Biol. Sci.*, 276 (1658): 987-991.

Humbert W, 1978. Cytochemistry and X-ray microprobe analysis of the midgut of *Tomocerus minor* Lubbock (Insecta, Collembola) with special reference to the physiological significance of the mineral concretions. *Cell Tissue Res.*, 187(3): 397-416.

Humbert W, 1979. The midgut of *Tomocerus minor* Lubbock (Insecta, Collembola): ultrastructure, cytochemistry, ageing and renewal during a moulting cycle. *Cell Tissue Res.*, 196(1): 39-57.

Ishii Y, Matsuura Y, Kakizawa S, Nikoh N, Fukatsu T, 2013. Diversity of bacterial endosymbionts associated with *Macrosteles* leafhoppers vectoring phytopathogenic phytoplasmas. *Appl. Environ. Microbiol.*, 79(16): 5013-5022.

Li Q, Zhong H, Zhang Y, Wei C, 2015. Comparative morphology of the distal segments of Malpighian tubules in cicadas and spittlebugs, with reference to their functions and evolutionary indications to Cicadomorpha (Hemiptera: Auchenorrhyncha). *Zool. Anz.*, 258: 54-68.

Licent PE, 1912. Recherches d'anatomie et de physiologie comparees sur le tube digestif des Homopteres superieures. *La Cellule*, 28: 6-161.

Marshall AT, 1964a. Spittle-production and tube-building by cercopoid nymphs (Homoptera). 1. The cytology of the Malpighian tubules of spittle-bug nymphs. *Quart. J. Micr. Sci.*, 105(70): 257-262.

Marshall AT, 1964b. Spittle production and tube-building by cercopoid nymphs (Homoptera). 2. The cytology and function of the granule zone of the Malpighian tubules of tube-building nymphs. *Quart. J. Micr. Sci.*, 105(72): 415-422.

Marshall AT, 1965. Spittle production and tube-building by cercopoid nymphs (Homoptera). 3. The cytology and function of the fibril zone of the Malpighian tubules of tubebuilding nymphs. *J. Cell Sci.*, 3(73): 37-44.

Marshall AT, 1966. Histochemical studies on a mucocomplex in the Malpighian tubules of cercopid larvae. *J. Insect Physiol.*, 2(8): 925-932.

Marshall AT, Cheung WWK, 1974. Studies on water and ion transport in homopteran insects: ultrastructure and cytochemistry of the cicadoid and cercopoid Malpighian tubules and filter chamber. *Tissue Cell*, 6 (1): 153-171.

McCutcheon JP, McDonald BR, Moran NA, 2009a. Convergent evolution of metabolic roles in bacterial co-symbionts of insects. *Proc. Natl. Acad. Sci. USA*, 106(36): 15394-15399.

McCutcheon JP, McDonald BR, Moran NA, 2009b. Origin of an alternative genetic code in the extremely small and GC-rich genome of a bacterial symbiont. *PLoS Genet.*, 5(7): e1000565.

Min KT, Benzer S, 1997. *Wolbachia*, normally a symbiont of *Drosophila*, can be virulent, causing degeneration and early death. *Proc. Natl. Acad. Sci. USA*, 94(20): 10792-10796.

Moran NA, 2007. Symbiosis as an adaptive process and source of phenotypic complexity. *Proc. Natl. Acad. Sci. USA*, 104(Suppl. 1): 8627-8633.

Moran NA, McCutcheon JP, Nakabachi A, 2008. Genomics and evolution of heritable bacterial symbionts. *Annu. Rev. Genet.*, 42: 165-190.

Moran NA, Tran P, Gerardo NM, 2005. Symbiosis and insect diversification: an ancient symbiont of sap-feeding insects from the bacterial phylum Bacteroidetes. *Appl. Environ. Microbiol.*, 71 (12): 8802-8810.

Noda H, Watanabe K, Kawai S, Yukuhiro F, Miyoshi T, Tomizawa M, Koizumi Y, Nikoh N, Fukatsu T, 2012. Bacteriome-associated endosymbionts of the green rice leafhopper *Nephotettix cincticeps* (Hemiptera: Cicadellidae). *Appl. Entomol. Zool.*, 47(3): 217-225.

Nunes PH, Camargo-Mathias MI, 2006. Ultrastructural study of the salivary glands of the sugarcane spittlebug *Mahanarva fimbriolata* (Stål, 1854) (Euhemiptera: Cercopidae). *Micron*, 37(1): 57-66.

O'Donnell MJ, Maddrell SH, 1983. Paracellular and transcellular routes for water and solute movements across insect epithelia. *J. Exp. Biol.*, 106(1): 231-253.

Radford SV, Misch DW, 1971. The cytological effect of ecdysterone on the midgut cells of the flesh-fly *Sarcophaga bullata*. *J. Cell. Biol.*, 49(3): 702-711.

Rakitov RA, 2002. Structure and function of the Malpighian tubules, and related behaviors in juvenile cicadas: evidence of homology with spittlebugs (Hemiptera: Cicadoidea & Cercopoidea). *Zool. Anz.*, 241(2): 117-130.

Redak RA, Purcell AH, Lopes JR, Blua MJ, Mizell RF, Andersen PC, 2004. The biology of xylem fluid-feeding insect vectors of *Xylella fastidiosa* and their relation to disease epidemiology. *Annu. Rev. Entomol.*, 49(1): 243-270.

Rost MM, 2006. Ultrastructural changes in the midgut epithelium in *Podura aquatica* L. (Insecta, Collembola, Arthropleona) during regeneration. *Arthropod Struct. Dev.*, 35(1): 69-76.

Rost-Roszkowska MM, Poprawa I, Swiatek P, 2007. Ultrastructural changes in the midgut epithelium of the first larva of *Allacma fusca* (Insecta, Collembola, Symphypleona). *Invertebr. Biol.*, 126(4): 366-372.

Sacchi L, Genchi M, Clementi E, Bigliardi E, Avanzati AM, Pajoro M, Negri I, Marzorati M, Gonella E, Alma A, Daffonchio D, Bandi C, 2008. Multiple symbiosis in the leafhopper *Scaphoideus titanus* (Hemiptera: Cicadellidae): details of transovarial transmission of *Cardinium* sp. and yeast-like endosymbionts. *Tissue Cell*, 40(4): 231-242.

Wright KA, Newell IM, 1964. Some observations on the fine structure of the midgut of the mite *Anystis* sp. *Ann. Entomol. Soc. Am.*, 57 (6): 684-693.

Zhong H, Wei C, Zhang Y, 2013a. Gross morphology and ultrastructure of salivary glands of the mute cicada *Karenia caelatata* Distant (Hemiptera: Cicadoidea). *Micron*, 45: 83-91.

Zhong H, Zhang Y, Wei C, 2013b. Anatomy and fine structure of the alimentary canal of the spittlebug *Lepyronia coleopterata* (L.) (Hemiptera: Cercopoidea). *Arthropod Struct. Dev.*, 42(6): 521-530.

合哑蝉和鞘翅圆沫蝉马氏管形态及超微结构比较研究

钟海英[1,2]，张雅林[1]，魏　琮[1,*]

（1. 西北农林科技大学昆虫博物馆，植保资源与病虫害治理教育部重点实验室，陕西杨凌 712100；2. 浙江省农业科学院植物保护与微生物研究所，浙江省植物有害生物防控重点实验室——省部共建国家重点实验室培育基地，杭州 310021）

摘要：【目的】本研究通过形态及超微结构比较研究，进一步了解蝉次目蝉总科和沫蝉总科这两个近缘类群的马氏管形态和功能分化。【方法】利用光学显微镜和透射电子显微镜技术，对合哑蝉 *Karenia caelatata* Distant 和鞘翅圆沫蝉 *Lepyronia coleoptrata* (Linnaeus)成虫马氏管的整体形态构造和超微结构进行了比较研究。【结果】结果显示，在整体形态构造方面，合哑蝉和鞘翅圆沫蝉马氏管在滤室外的部分均分化为 4 个区段：过渡区、基部区、端部区和末端区。合哑蝉马氏管末端区附着于回肠并被结缔组织包裹，最末端两两愈合为一体；鞘翅圆沫蝉的末端区不愈合，最末端膨大成棒状并贴附于直肠。超微结构观察表明，这两种蝉次目昆虫的马氏管管壁细胞均具有基膜内褶和微绒毛。合哑蝉马氏管基部区管壁细胞含有分泌囊泡、分泌颗粒、内质网和矿物质颗粒。鞘翅圆沫蝉马氏管基部区管壁细胞包含大量分泌囊泡，端部区细胞中的卵形囊泡与基膜愈合。马氏管中的微生物形态表明，合哑蝉马氏管的基部区、端部区中的微生物可能为共生细菌，鞘翅圆沫蝉马氏管的过渡区中微生物可能为病原菌。【结论】合哑蝉和鞘翅圆沫蝉马氏管在形态构造和超微结构方面的异同，一方面为蝉总科和沫蝉总科的姊妹群关系提供了重要证据，另一方面也为其马氏管的排泄及分泌功能分化研究提供了重要信息。相关微生物的发现为进一步探讨共生菌与蝉次目昆虫的协同进化，以及利用微生物进行该类害虫的防治提供了信息。

关键词：头喙亚目；合哑蝉；鞘翅圆沫蝉；排泄系统；共生细菌；透射电镜；光学显微镜

中图分类号：Q964　　**文献标识码：**A　　**文章编号：**0454-6296(2017)07-0825-10

（责任编辑：赵利辉）